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ORIGINAL RESEARCH PAPER

Microfungal diversity of *Juncus trifidus* L. and *Salix herbacea* L. at isolated locations in the Sudetes and Carpathian Mountains

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Abstract

During cold periods in the Pleistocene Epoch, many plants known as the "relict species" migrated and inhabited new areas. Together with plants, some microfungi also migrated, remaining present on plants and in plant communities. However, the relationship between fungi and the migrating plants (especially host plants) is not well understood. Therefore, we examined the diversity and distribution of microfungi associated with two migratory relict plants in the Sudetes and Carpathian Mountains: *Salix herbacea* L. and *Juncus trifidus* L. In total, we found 17 taxa of fungi that were collected from nine different locations. Nine fungal taxa were collected on *S. herbacea*, and eight taxa on *J. trifidus*. Localities richest of fungi on *S. herbacea* were Mały Śnieżny Kocioł (Karkonosze Mts, Sudetes) and on *J. trifidus*, the Tatra Mts (Carpathian Mts). This work provides new insights into the distribution of fungi inhabiting *S. herbacea* and *J. trifidus* in Poland.

Keywords

biodiversity; relict-associated microfungi; mountains; *Juncus*; *Salix*; Central Europe

Introduction

The present distribution and genetic structure of organisms are consequences of repeated climatic changes in the Pleistocene during the past several million years. The cold periods in the Pleistocene Epoch caused substantial changes in ecosystems, such as organism extinction, habitat fragmentation, and modified vegetation in the colder areas of Europe, America, and the Arctic [1–4].These climatic changes were also the primary reason for numerous plant or animal migrations to the southern part of Europe or into warmer localities, in front of glaciers, where they were able to survive unfavorable conditions; once favorable conditions returned, they could migrate back to the northern areas [5–7]. Such organisms, commonly called glacial relicts, were widely distributed when the climate changed in the Holocene Epoch; the changing climate also caused numerous organisms to adapt to the new climatic conditions and reduced their ranges to smaller refugia, mostly in high mountains, where the arctic boreal conditions and cold climate remained. It is possible that together with relict plant species, some microfungal communities migrated and inhabited the relicts in new areas. Although the distributional data from various localities have been published, the occurrence and distribution of microfungi on relict plants is still poorly understood [1,8]. According to Chlebicki and other authors [1,8–15], 35 microfungal species have been found so far on various parts of *Salix herbacea* shoots, and almost all were collected from small and scarce plant branches, especially from the leaves of a dwarf willow. In general, from the reported 171 fungi taxa occurring on plants from the genus *Juncus* [16], Šandová and Chlebicki as well as other authors listed 32 microfungal species from *J. trifidus* [1,17–21]. Some of these species can inhabit not only *J. trifidus* but also other species from Juncaceae or Cyperaceae. The study presents new data on the distribution of fungal species inhabiting the relict plants *J. trifidus* and *S. herbacea* in the Sudetes and Carpathian Mts. These results could also better our understanding about the coexistence of and mutual relationships among fungi and plants.

Material and methods

The research was conducted in several locations in the Sudetes and Carpathian Mts ranges in summer 2017 and included specimens of *J. trifidus* and *S. herbacea* (Tab. 1, Tab. 2, Fig. 1). *Salix herbacea* is one of the smallest willows that can survive in harsh high mountains and boreal environments. It is an Arctic-Alpine plant species with an Amphi-Atlantic distribution [22]. It can be found in subarctic North America, Greenland, Iceland, Scotland, and northern Siberia. In Europe, this plant is common in Scandinavia, but also in the Alps and Pyrenees with a few localities in Apennines, Balkan Peninsula, the Sudetes (Karkonosze Mts, Hrubý Jeseník), and in the Carpathian Mountains (Pilsko Mt, Tatra Mts) [23]. Dwarf willow can be found in alpine or subalpine zones on rocks, in rock crevices, snow beds, or in marshes, usually on acid rock substrates, from 1,400 to about 2,200 m a.s.l. The localities of collected samples were marked on the map (Fig. 1) by subscript "Xa".

According to Hulten [22], *J. trifidus* is Amphi-Atlantic plant, native to boreal regions of Northern Canada, Northeastern United States, Iceland, Greenland, and northern regions of Britain and Siberia. In Europe, it is also common in Scandinavia and present in Pyrenees, Alps, Apennines, and Balkan Peninsula. In Poland, *J. trifidus* occurs in scattered localities in alpine and subalpine zones in the Sudetes (Karkonosze Mts, Śnieżnik Mt, Hrubý Jeseník) and Carpathian Mts (Babia Góra Mt, Tatra Mts) from 1,300 to about 2,200 m a.s.l. [23]. Chlebicki [1] noted that 18 taxa of fungi can be found on different plant species from the family Cyperaceae (genera *Carex*, *Juncus*, and *Luzula*). The localities of collected samples on *J. trifidus* were marked on the map in Fig. 1 by subscript "Xb".

The occurrence of fungi on stems, leaves, inflorescences, fruits, bracts, and bracteoles has been analyzed. Plant parts were taken to the laboratory and after being immersed in a disinfectant, they were placed on standard PDA medium in Petri dishes. After growth, fungi were identified using a light microscope (ZeissAxiophot, Carl Zeiss, Jena, Germany) at a magnification of 400×. For some morphological structures, i.e.,

Tab. 1 Locations of studied *S. herbacea* populations.

No.	Location	Altitude (m)	Coordinates	Comments
		Karkonosze Mts		
1a	Mały Śnieżny Kocioł	1,477	50°46′46.38″ N 15°33′23.41″ E	Small patch above couloir
		Beskid Mts		
2a	Pilsko Mt	1,492	49°31′41.16″ N 19°18′46.85″ E	At the top, plants on rock walls
		Tatra Mts		
3a	Nižné Wahlenbergovo pleso	1,805	49°8′52.77″ N 20°1′50.5″ E	On the rocks
4a	Furkotský štít	1,772	49°8′47.21″ N 20°1′45.5″ E	On the rocks

Tab. 2 Locations of studied *J. trifidus* populations.

No.	Location	Altitude (m)	Coordinates	Comments
		Karkonosze Mts		
1b	Śnieżka Mt	1,537	50°44′12.01″ N 15°44′34.97″ E	Commonly on the ground and rocks
2b	Czarny Grzbiet	1,419	50°44′27.78″ N 15°45′5.76″ E	Close to Śnieżka Mt
		Babia Góra massif		
3b	Diablak	1,692	49°34′26.21″ N 19°32′0.72″ E	Commonly on the ground
		Tatra Mts		
4b	Chuda Turnia	2,027	49°14′2.06″ N 19°54′56.92″ E	On the ground and rocks
5b	Ciemniak Mt	2,050	49°13′49.88″ N 19°54′11.89″ E	On the ground and rocks
6b	Nižné Wahlenbergovo pleso	1,894	49°9′5.81″ N 20°1′43.98″ E	Commonly on the ground and rocks
7b	Furkotský štít	1,781	49°8′48.93″ N 20°1′42.68″ E	Commonly on the rocks

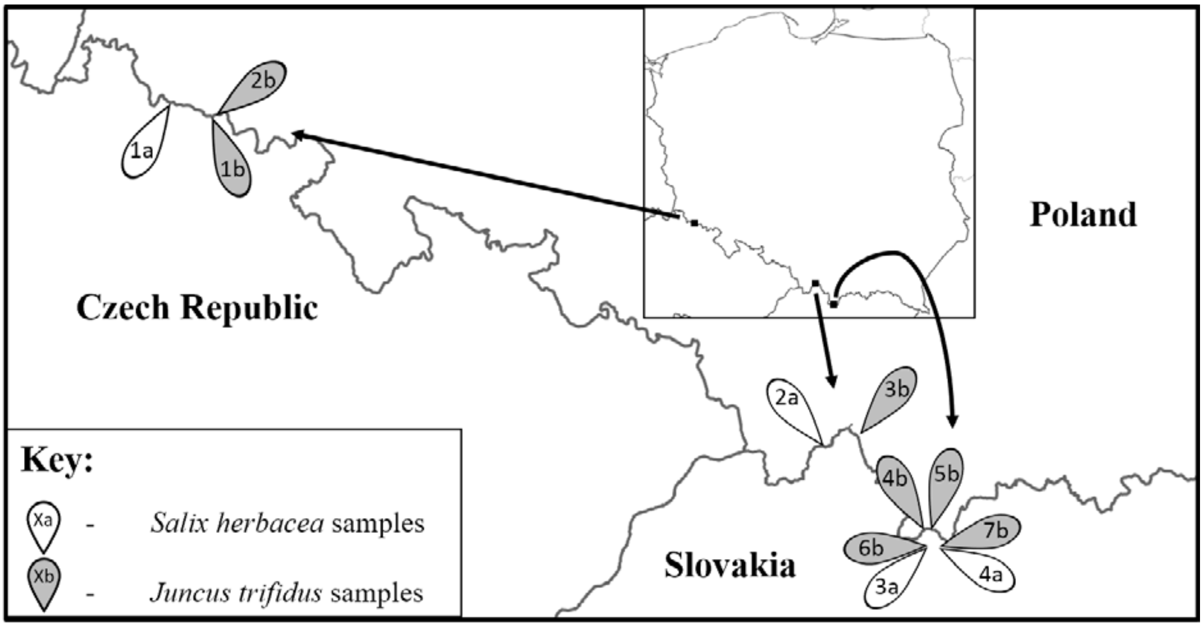

Fig. 1 Locations of collected samples.

ornamentation, a magnification of 1,000× with immersion oil was used. All samples were collected, and the species were determined by Brayan Jacewski. Fungi species were identified using specialized bibliography [24–29]. Taxonomic nomenclature of fungi follows that used in the Index Fungorum [30], and plant names were given according to Mirek et al. [31]. In a few cases, molecular methods were used for species determination. The fungal DNA was extracted using a common Doyle and Doyle [32] method of nucleic acid extraction with CTAB. ITS rDNA regions were used for species determination with the primer pairs ITS1F and ITS4 [33]. The detailed procedure of PCR reaction and temperature parameters were the same as those described by Pusz and Urbaniak [8]. Sequencing, postreaction purification, and reading were performed by a sequencing service (Genomed S.A., Warsaw, Poland), using an ABI377XL Automated DNA Sequencer (Applied Biosystems, Carlsbad, CA, USA). The sequences were

Tab. 3 Fungi collected on *S. herbacea*.

Mały Śnieżny Kocioł
Alternaria alternata
Alternaria infectoria
Aspergillus brasiliensis
Ceuthospora spp.
Cladosporium allicinum
Melampsora arctica
Penicillium notatum
Pilsko Mt
Alternaria alternate
Alternaria infectoria
Ceuthospora spp.
Cladosporium allicinum
Penicillium notatum
Pestalotiopsis spp.
Truncatella angustata
Nižné Wahlenbergovo pleso
Alternaria alternate
Penicillium notatum
Furkotský štít
Alternaria alternata
Cladosporium allicinum
Penicillium notatum

analyzed with FinchTV [34] and MEGA 5.0 [35]. Species determinations were verified using BLAST software [36]. Collected samples were deposited in the Herbarium of the Museum of Natural History in Wroclaw (WRSL) with numbers from JBr-2017-0001 to JBr-2017-0038 (Tab. 4).

Results

In this study, 17 taxa of fungi were collected from nine localities: eight taxa on *S. herbacea* and eight on *J. trifidus*. All detected microfungal species associated with the two glacial relicts are presented in Tab. 3 and Tab. 4. Current results were also compared with previously published data (Tab. 5, Tab. 6). The localities richest in fungi on *S. herbacea* were Mały Śnieżny Kocioł (Karkonosze Mts, Sudetes) and on *J. trifidus*, the Tatra Mts (Carpathian Mts). Detailed descriptions of all the findings are given below.

Fungi collected on *S. herbacea*

Ascomycota

Alternaria alternate (Fr.) Keissl.

Description. Conidiophores 50 × 3–6 µm pale brown to olive brown, forming bushy heads consisting of 4–8 conidial chains. Conidia 20–63 × 9–18 µm, obclavate, with short conical beak at the tip, pale brown to light brown. GenBank accession numbers: MH118270, MH118271.

Habitat. Dead leaves, petioles.

Collected material. Above Mały Śnieżny Kocioł (Karkonosze Mts, July 20, 2017; JBr-2017-0001), at the top of Pilsko Mt (Beskid Mts, August 30, 2017; JBr-2017-0008), near Nižné Wahlenbergovo pleso (Tatra Mts, September 2, 2017; JBr-2017-0015), Furkotský štít (Tatra Mts, September 2, 2017; JBr-2017-0017).

Comments. Common fungus causing leaf spots, blights, and other symptoms on many dicotyledonous plants. Ubiquitous species that usually disperses in the air [15].

Alternaria infectoria E. G. Simmons

Description. Colonies pale grey to silvery with white and fluffy aerial mycelia. Conidiophores 11–21 µm long. Conidia 20–27.5 × 7.5–16 µm, obclavate, in long or short chains, predominantly without longitudinal septa. GenBank accession number: MH118273.

Habitat. Dead leaves and petioles.

Collected material. Above Mały Śnieżny Kocioł (Karkonosze Mts, July 20, 2017; JBr-2017-0002), on the top of Pilsko Mt (Beskid Żywiecki, August 30, 2017; JBr-2017-0009).

Comments. Species from the Pleosporales order, ubiquitous, saprotrophic species which could be associated with seeds [37]. It was identified, for example, in Australia on *Tanacetum cinerariifolium* Trevir. leaves and dead flower stems (causing small black spots), and in the UK on *Triticum aestivum* L. [38,39].

Aspergillus brasiliensis Varga, Frisvad & Samson

Description. Conidiophores 900–1,200 µm long, unbranched, enlarged at the tip, forming swollen vesicles. Stipes hyaline. Conidia globose to sub-globose, 3.5–5 µm in diam., dark brown to black.

Tab. 4 Fungi collected on *J. trifidus*.

Śnieżka Mt
Arthrinium cuspidatum
Cladosporium herbarum
Phomatospora dinemasporium
Czarny Grzbiet
Arthrinium cuspidatum
Botrytis cinereal
Cladosporium herbarum
Periconia atra
Diablak
Botrytis cinerea
Periconia atra
Unguicularia costata
Chuda Turnia
Botrytis cinerea
Cladosporium herbarum
Periconia atra
Phaeosphaeria vagans
Ciemniak Mt
Cladosporium herbarum
Nimbomollisia eriophori
Nižné Wahlenbergovo pleso
Cladosporium herbarum
Unguicularia costata
Furkotský štít
Phaeosphaeria vagans

Habitat. Dead leaves and petioles placed on PDA medium.

Collected material. Above Mały Śnieżny Kocioł (Karkonosze Mts, July 20, 2017; JBr-2017-0003).

Comments. Cosmopolitan, saprobic species, widespread in the environment.

Ceuthospora sp.

Description. Colonies black, leathery, rounded or oval 0.5 mm in diam., with one locule. Conidia 12–16 × 2–4 μm, subcylindrical, hyaline, with funnel-shaped apical appendages.

Habitat. Bottom side of dead leaves.

Collected material. Above Mały Śnieżny Kocioł (Karkonosze Mts, July 20, 2017; JBr-2017-0004), at the top of Pilsko Mt (Beskid Żywiecki, August 30, 2017; JBr-2017-0010).

Comments. Widespread fungal genus, usually causing leaf spots. Sutton [29] listed its occurrence in association with dicotyledonous plants. Many species of this genus are plant pathogens, i.e., *C. lauri* and *C. innumera* parasitize *Eucalyptus* leaves [40], and *C. phaeocomes* parasitizes holly leaves [41].

Cladosporium allicinum (Fr.) Bensch, U. Braun & Crous

Description. Colonies olivaceous to dark brown, powdery. Conidiophores 30–200 μm long, 3–6 μm wide, unbranched, light brown, with characteristic, long secondary ramoconidia. Conidia ovoid to lemon-shaped 4–6 × 3 μm, unicellular, pale to dark brown with dark hila, forming chains at the end of conidiophores, with prominent ornamentation [34]. GenBank accession number: MH118272.

Habitat. Dead leaves and petioles.

Collected material. Above Mały Śnieżny Kocioł (Karkonosze Mts, July 20, 2017; JBr-2017-0005), at the top of Pilsko Mt (Beskid Mts, August 30, 2017; JBr-2017-0011), Furkotský štít (Tatra Mts, September 2, 2017; JBr-2017-0018).

Comments. Species distributed worldwide, but not commonly isolated. Present on leaves and fruits of dicotyledons as a secondary saprotrophic infection on decaying parts [24]. In Germany, this species was isolated from various samples, including leaves of *Robinia pseudoacacia* L., *Acer campestre* L., and *Alnus glutinosa* (L.) Gaertn. [42].

Penicillium notatum Westling

Description. Colonies: at first yellow-green changing into dark green, velvety, subtle floccose. Conidiophores 250–500 μm, mononematous, two to three branches, hyaline. Metulae cylindrical with three–six flask-shaped phialides. Conidia globose to subglobose, hyaline, 3–4 μm in diam.

Habitat. Dead leaves and petioles placed on PDA medium.

Collected material. Above Mały Śnieżny Kocioł (Karkonosze Mts, July 20, 2017; JBr-2017-0007), at the top of Pilsko Mt (Beskid Mts, August 30, 2017; JBr-2017-0012), near Nižné Wahlenbergovo pleso (Tatra Mts, September 2, 2017; JBr-2017-0016), Furkotský štít (Tatra Mts, September 2, 2017; JBr-2017-0019).

Comments. Species common worldwide, inhabiting moist soils; spores present in the air. There are two other species of *Penicillium* genus associated with *S. herbacea*: *P. citrinum*, which was reported in the Apennines and on Surtsey island, and *P. palitans*, which was collected also on Surtsey island [9,43]. All mentioned species likely belong to different sections: *P. notatum* belongs to the *Chrysogena* section, wherein colonies of species are characterized by having a velvety texture and producing penicillin (majority). *Penicillium citrinum* is assigned to sect. *Citrina*. The majority of species found in this section form symmetrical biverticillate conidiophores with flask-shaped phialides and small conidia. *Penicillium palitans* belongs to sect. *Fasciculata* with most species having granulose or fasciculate colony texture [44–46].

Pestalotiopsis sp.

Description. Conidiomata acervular. Conidiophores 10–15 × 2 μm, hyaline, cylindrical. Conidia 25 × 6 μm, four–six-celled; pigmentation can be affected by environmental conditions.

Habitat. Twigs.

Collected material. Pilsko Mt (Beskid Mts, August 30, 2017; JBr-2017-0013).

Comments. Species of the genus *Pestalotiopsis* are common plant pathogens, which are not highly host-specific. They are often isolated as endophytes or as saprobes [47,48]. They may also cause grey blight disease of *Persea* spp. [49]. Most species are distinguished by conidia size, as well as by the length and the number of apical appendages [29].

Truncatella angustata (Pers.) S. Hughes

Description. Conidiomata acervular. Conidia four-celled, 16–19 × 7–9 μm, slightly curved, with two dark brown, median cells, with two hyaline apical appendages.

Habitat. Dead petioles.

Collected material. Pilsko Mt (Beskid Mts, August 30, 2017; JBr-2017-0014).

Comments. Cosmopolitan species that is usually found on leaf spots on different genera of Ericaceae, Oleaceae, Rosaceae, and Salicaceae. The species was identified on *Malus domestica* Borkh. in the Netherlands, on *Rosa canina* L. in Kazakhstan, and on blueberry twigs (*Vaccinium* spp.) [50–52].

Basidiomycota

Melampsora arctica Rostr.

Description. Uredinia intense yellow to pale orange, circular, mainly epiphyllous, pulverulent. Urediniospores globoid, 15–20 × 17–25 μm. Paraphyses 50–60 μm long, intermixed with urediniospores, colourless wall, 5–7 μm thick at the base and 3–5 μm thick at the top, rarely verrucose.

Habitat. Leaves (dead and alive).

Collected material. Above Mały Śnieżny Kocioł (Karkonosze Mts, July 20, 2017; JBr-2017-0006).

Comments. The data on the distribution of *M. arctica* summarized by Chlebicki [1] indicate that this species is relatively often found on *S. herbacea*. It was reported in both Polish and Slovakian parts of the Tatra Mountains [53]. *Melampsora arctica* is considered as an Arctic-Alpine species, and it was also noticed on other *Salix* species, e.g., *S. lapponum* or *S. hastata*, growing in Arctic-Alpine areas and in cold climates. This confirms the older data concerning the occurrence of *M. arctica* in the Alps, Scandinavia, Greenland, and North America [10,54].

Fungi collected on *J. trifidus*

Ascomycota

Arthrinium cuspidatum (Cooke & Harkn.) Tranzschel

Description. Colonies black. Conidiophores 20–25 µm long, hyaline, with dark septa. Conidia 13–15 × 8–10 µm, one-celled, curved, brown, double-horned.

Habitat. Dead stems and leaves.

Collected material. Śnieżka Mt (Karkonosze Mts, July 20, 2017; JBr-2017-0020); Czarny Grzbiet (Karkonosze Mts, July 20, 2017; JBr-2017-0023).

Comments. This species was previously reported to inhabit *Juncus* and *Carex* species in the Eastern Alps, North America, and Scandinavia, and was collected in the Tatra and Karkonosze Mts [1]. In Scandinavia, the species was noted in Abisko Östra. Šandová [17,18] found *A. cuspidatum* on *J. trifidus* in the Czech Republic in Šumava, Karkonosze, and Hrubý Jeseník Mts. In the Czech Republic, this species seems to be common on *Juncus* spp. in subalpine and alpine belts [1,17,20].

Botrytis cinerea Pers.

Description. Conidiophores 1,500–2,000 × 15–20 µm, brown, highly branched. Conidia 10–16 × 6–9 µm, obovoid to ellipsoid, pale brown.

Habitat. All above-ground parts of a studied plant.

Collected material. Czarny Grzbiet; host growing on rocks (Karkonosze Mts, July 20, 2017; JBr-2017-0024); Diablak; host growing on rocks (Babia Góra massif, September 1, 2017; JBr-2017-0027); Chuda Turnia, *Junco trifidi-Festucetum airoidis* plant community (Tatra Mts, September 1, 2017; JBr-2017-0030).

Comments. Cosmopolitan, necrotrophic species, mostly growing on living and dead dicotyledonous plants. Most notable hosts are wine grapes and strawberry fruits. Brown lesions develop on twigs, slowly causing withering by breaching the plant cuticle and producing a wide range of metabolites [55]. Šandová [18] found *B. cinereal* in the Czech Republic in several mountain ranges: Šumava, Karkonosze Mts, Králický Sněžník, and Hrubý Jeseník Mts, suggesting that *B. cinerea* is not especially common on *J. trifidus*, but more frequently on other *Juncus* species.

Cladosporium herbarum (Pers.) Link

Description. Conidiophores 80–120 µm, long brown, erect. Conidia 6–10 × 3–5 µm, in branching chains, elliptical to cylindrical, thick walled, dark brown, no- or one-septate.

Habitat. Dead stems, leaves, and bracts.

Collected material. Śnieżka Mt (Karkonosze Mts, July 20, 2017; JBr-2017-0021); Czarny Grzbiet (Karkonosze Mts, July 20, 2017; JBr-2017-0025); Chuda Turnia (Tatra Mts, September 1, 2017; JBr-2017-0031); Ciemniak Mt (Tatra Mts, September 1, 2017; JBr-2017-0034); Nižné Wahlenbergovo pleso (Tatra Mts, September 2, 2017; JBr-2017-0036).

Comments. Cosmopolitan species, growing on many species of plants [19]. From *J. trifidus*, *C. herbarum* was reported in the Czech Republic [17,56].

Phomatospora dinemasporium J. Webster

Description. Conidiomata acervular, black, up to 200 µm in diam., superficial, densely setose. Setae up to 220 µm long, dark. Conidiophores 25 × 2 µm, hyaline. Conidia 9–11 × 1–2 µm, cylindrical.

Habitat. Dead leaves and bracts.

Collected material. Śnieżka Mt (Karkonosze Mts, July 20, 2017; JBr-2017-0022).

Comments. Common saprobic species, growing on plants from Poaceae and Juncaceae [57]. The species was found on leaves of *Populus tremula* L. in Germany, and on blades of *Secale cereale* L. [58].

Nimbomollisia eriophori (L. A. Kirchn.) Nannf.

Description. Apothecia turbinate to discoid, sessile, 250–400 µm in diam., light brownish. Excipulum textura globosa, cells 10 µm at base, brown at flanks, light brown at the base and edge. Asci 70–80 × 12–15 µm, clavate. Spores 15 × 5 µm, fusiform, with a thin gelatinous coat. Paraphyses cylindrical, branched, segmented, with circinate tips.

Habitat. Dead stems.

Collected material. Ciemniak Mt (Tatra Mts, September 1, 2017; JBr-2017-0035).

Comments. Common saprobic species growing on various species of Cyperaceae and Juncaceae. Previously, this species was identified by Šandová on *J. trifidus* [18] and *J. filiformis* [59]. Müller and Défago [60] found this species on *Eriophorum angustifolium* Honck., *E. scheuchzeri* Hoppe and *Carex fusca* Bell et al.

Periconia atra Corda

Description. Conidiophores 250–300 × 3–5 µm, brown, with a swollen apex. Conidia 5–10 µm in diam, brown, rounded.

Habitat. Dead stems, leaves, and bracts.

Collected material. Czarny Grzbiet (Karkonosze Mts, July 20, 2017; JBr-2017-0026) Diablak (Babia Góra massif, September 1, 2017; JBr-2017-0028) Chuda Turnia (Tatra Mts, September 1, 2017; JBr-2017-0032).

Comments. Common saprobic species growing on dead leaves of various species of Cyperaceae, Juncaceae, and Poaceae [19]. The species was identified on *Carex pendula* Huds., *C. riparia* Curtis, *Juncus* sp. [61–63], and *J. trifidus* [18].

Phaeosphaeria vagans (Niessl) O. E. Erikss.

Description. Pycnidia 200–250 × 400–450 µm in diam., dark brown to black, ellipsoidal. Asci 135 × 20 µm, cylindrical. Ascospores 20–24 × 8–9 µm, six-celled, slightly curved.

Habitat. Dead stems.

Collected material. Chuda Turnia (Tatra Mts, September 1, 2017; JBr-2017-0033); Furkotský štít (Tatra Mts, September 1, 2017; JBr-2017-0038).

Comments. Common saprobic species growing on dead stems of species from Poaceae, Cyperaceae, and Juncaceae [17]. The species was found on dried grass culms in British Columbia (Canada) by Ceska and Ceska [64], and on plants from *Agrostis*, *Calamagrostis*, *Festuca*, and *Lolium* [65].

Unguicularia costata (Boud.) Dennis

Description. Apothecia 150–200 μm in diam., 150 μm high, pale white, urn-shaped, sessile, superficial. Ascopores 9–13 × 2–2.5 μm, straight, hyaline.

Habitat. Dead stems.

Collected material. Diablak (Babia Góra massif, September 1, 2017; JBr-2017-0029); Nižné Wahlenbergovo pleso (Tatra Mts, September 2, 2017; JBr-2017-0037).

Comments. The rarest of the identified species. Growing on dicotyledonous plants. Not common on *J. trifidus* [17]. This species was identified on *J. effusus* L. in the Czech Republic [66].

Discussion

No fungal species exclusive to *S. herbacea* were found during this study. The occurrence of *Melampsora arctica*, the truly Arctic-Alpine species, above Mały Śnieżny Kocioł (Karkonosze Mts) confirmed the subarctic characteristic of *S. herbacea* and geographical connections of disjunctive localities that link the Alps, Carpathian Mts, Scandinavia, and Greenland (Tab. 5). *Melampsora arctica* has been also found in the Tatra Mts. (Carpathian Mts) by Chlebicki [1]. During this study, the presence of *Alternaria infectoria* (Mały Śnieżny Kocioł, Pilsko Mt) and *Cladosporium allicinum* (Mały Śnieżny Kocioł, Pilsko Mt, Furkotský štít) was noted for the first time on *S. herbacea*. The occurrence of *Alternaria alternata* – a species previously noted on *S. herbacea* in the Karkonosze Mts only by Pusz and Urbaniak [8] – has been confirmed in other dwarf willow populations (Mały Śnieżny Kocioł), as well as in new locations (Pilsko Mt, Furkotský štít, Nižné Wahlenbergovo pleso). Similarly, the occurrence of *Aspergillus brasiliensis* and *Ceuthospora* sp. in different locations in the Karkonosze Mts has been confirmed. *Ceuthospora* sp. has been observed for the first time in the Beskid Mts (Plisko Mt) [8]. The *Penicillium notatum* previously reported in the Apennines and Karkonosze Mts was again collected in Karkonosze Mts, and also found in the Tatra and Beskid mountains [8,9]. The species in the *Pestalotiopsis* genus and *Truncatella angustata*, both previously reported on *S. herbacea* only in the Karkonosze Mts, were reported in the Pilsko Mt (Beskids Mts) [8].

Among the numerous fungal species inhabiting *J. trifidus*, some (i.e., *Lophodermium juncinum* and *Naeviella paradoxa*) were noted to exist exclusively on *J. trifidus* [1,18], whereas other species such as *Arthrinium cuspidatum*, *Ascochyta junci*, or *Leptosphaeria sepalorum* strictly inhabit various *Juncus* species, not only *J. trifidus* [17]. However, the occurrence of the mentioned oligophagous fungi species was not confirmed in the present study. *Lophodermium juncinum* has not been reported in Poland so far, with the closest known locations in the Eastern Alps (Tab. 6). *Naeviella paradoxa* has been noted in the Polish mountains (Karkonosze and Tatra Mts) only by Chlebicki [1]. The occurrence of *Arthrinium cuspidatum*, *Botrytis cinerea*, and *Cladosporium herbarum* has been confirmed in the locations studied in the Karkonosze Mts [17]. *Botrytis cinerea* was noted in the Tatra Mts and Babia Góra massif for the first time. The new, uncharted locations of *Nimbomollisia eriophori* and *Phaeosphaeria vagans* were found on *J. trifidus* in the Tatra Mts, and in the case of *Periconia atra*, also at Pilsko Mt.

In summary, the total number of fungi noticed during our research, as well as reported in previously on *S. herbacea* is 56, and on *J. trifidus* 98 species. In the case of *S. herbacea*, the most fungi-rich localities were the mountain ranges of the Karkonosze, Tatra, and Apennines Mts. Certainly, this species is connected with small, but quite numerous and widespread communities of *S. herbacea* especially in the Tatra Mts. The past 150 years of exploration of the Karkonosze Mts have also contributed to the relatively thorough knowledge of mountain mycobiota [21]. Likewise, the relatively broad knowledge of fungal biota associated with *J. trifidus* in the Karkonosze Mts may be the result of recent intensive, multiyear research. It is also fostered by the extensive *J. trifidus* populations. This subalpine species often covers areas that stretch for several kilometers of mountains peaks. It can be concluded that the mycobiota of both studied plant relicts seems to be highly diverse. However, according to results of Pusz and Urbaniak [8], detailed analyses

Tab. 5 Fungi species reported on *S. herbacea* based on own observations and literature data.

Species	Locality: Apennine Mts	Karkonosze Mts	Tatra Mts	Beskid Mts	Alps	Khibiny Mts	Greenland	Rila Mts	Retezat Mts	Chornohora Mts	Rodna Mts	References
Acremonium murorum (Corda) W. Gams	×											[9]
Alternaria alternata (Fr.) Keissl.	×	×*	*	*								[8,9]
Alternaria infectoria E. G. Simmons		*		*								
Aspergillus brasiliensis Varga, Frisvad & Samson		×*										[8]
Ceuthospora spp.		×*		*								[8]
Cladosporium allicinum (Fr.) Bensch, U. Braun & Crous		*	*	*								
Cladosporium cladosporioides (Fresen.) G. A. de Vries	×	×										[8,9]
Cladosporium oxysporum Berk. & M. A. Curtis	×											[9]
Colletotrichum spp.		×										[8]
Botrytis cinerea Pers.	×	×										[8,9]
Epicoccum nigrum Link	×											[9]
Gibberella avenacea R. J. Cook		×										[8]
Gibberella baccata (Wallr.) Sacc.		×										[8]
Gibberella intricans Wollenw.		×										[8]
Lecanicillium lecanii (Zimm.) Zare & W. Gams	×											[9]
Lecanicillium longisporum (Petch) Zare & W. Gams	×											[9]
Linospora arctica P. Karst.					×							[14]
Melampsora arctica Rostr.		*	×		×		×					[1]
Melampsora epitea Thüm.								×	×			[11,12]
Mucor hiemalis Wehmer	×											[9]
Mycosphaerella maculicola (G. Winter) Tomilin										×		[1]
Mycosphaerella salicicola (Fuckel) Johanson ex Oudem.							×					[10]
Penicillium citrinum Thom	×	×										[8,9]
Penicillium notatum Westling	×	×*	*	*								[8,9]
Pestalotia spp.	×											[9]
Pestalotiopsis spp.		×		*								[8]
Phleospora sp.			×									[1]
Phoma leveillei Boerema & G. J. Bollen		×										[8]
Pleuroceras insulare (Johanson) M. Monod					×							[14]
Pleuroceras groenlandicum (Rostr.) M. E. Barr												[13]
Rhytisma salicinum (Pers.) Fr.		×	×					×			×	[1,11,67]
Strasseria geniculata (Berk. & Broome) Höhn.										×		[1]
Truncatella angustata (Pers.) S. Hughes		×		*								[8]
Venturia subcutanea Dearn.			×			×	×					[1,10]

× – species reported in literature;
* – species collected during this study.

Tab. 6 Fungi species reported on *J. trifidus* based on own observations and literature data.

Species	Locality										References
	Šumava Mts	Karkonosze Mts	Králický Sněžník	Hrubý Jeseník Mts	Ural Mts	Tatra Mts	Eastern Alps	Babia Góra massif	Chornohora Mts	Abisko region	
Arthrinium cuspidatum (Cooke & Harkn.) Tranzschel	×	×*	×	×		×	×				[1,17,18]
Arthrinium arundinis (Corda) Dyko & B. Sutton					×	×					[19]
Ascochyta caricicola Melnik						×					[1]
Ascochyta junci (Oudem.) Melnik		×		×							[17]
Aureobasidium pullulans (de Bary & Löwenthal) G. Arnaud						×					[19]
Botrytis cinerea Pers.	×	×*	×	×		*		*			[18]
Cistella fugiens (W. Phillips) Matheis					×		×				[1,21]
Cladosporium herbarum (Pers.) Link	×	×*	×	×		×*					[18,19]
Diplonaevia emergens (P. Karst.) B. Hein		×							×		[1]
Epicoccum nigrum Link	×	×		×							[18]
Hysteronaevia minutissima (Rehm) Nannf.	×						×		×		[1,18,21]
Hysteropezizella diminuens (P. Karst.) Nannf.	×	×	×	×			×			×	[1,18,21]
Lachnum calycioides (Rehm) Rehm	×	×	×	×	×	×	×		×	×	[1,18,21]
Lachnum diminutum (Roberge ex Desm.) Rehm		×									[17]
Lachnum roseum (Rehm) Rehm	×						×				[1,18]
Leptosphaeria sepalorum (Vleugel) Lind					×		×			×	[1]
Lophodermium juncinum (Jaap) Terrier							×				[1]
Mycosphaerella perexigua (P. Karst.) Johanson	×	×	×	×						×	[1,18]
Naeviella paradoxa (Rehm) Clem.		×				×	×				[1,18]
Nimbomollisia eriophori (L. A. Kirchn.) Nannf.	×	×	×	×		*					[17,18]
Penicillium expansum Link						×					[19]
Periconia atra Corda	×	×*	×			*		*			[18]
Phaeosphaeria juncicola (Rehm ex G. Winter) L. Holm					×		×		×		[1,21]
Phaeosphaeria vagans (Niessl) O. E. Erikss.				×		*					[17]
Phialocephala sp.				×							[17]
Phomatospora dinemasporium J. Webster	×	×*	×	×			×		×		[1,18,21]
Pseudoseptoria sp.	×	×		×							[17]
Pycnothyrium junci Grove	×			×							[17]
Septoria chanousiana Ferraris		×									[17]
Stagonospora junciseda (Sacc.) Mussat	×										[17]
Stagonospora caricinella Brunaud		×						×			[1]
Unguicularia costata (Boud.) Dennis				×		*		*			[17]

× – fungi reported in literature data;
* – fungi collected during this study.

still provide new data on the species diversity and the biogeography of fungal species, which justifies the need for further research in this direction.

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